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(54) **METHOD FOR ENHANCING THE HYBRIDIZATION EFFICIENCY OF TARGET NUCLEIC ACIDS USING A SELF-ADDRESSABLE, SELF-ASSEMBLING MICROELECTRONIC DEVICE**

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(57) **ABSTRACT**

A self-addressable, self-assembling microelectronic device is designed and fabricated to actively carry out and control multi-step and multiplex molecular biological reactions in microscopic formats. These reactions include nucleic acid hybridizations, antibody/antigen reactions, diagnostics, and biopolymer synthesis. The device can be fabricated using both microlithographic and micro-machining techniques. The device can electronically control the transport and attachment of specific binding entities to specific microlocations. The specific binding entities include molecular biological molecules such as nucleic acids and polypeptides. The device can subsequently control the transport and reaction of analytes or reactants at the addressed specific microlocations. The device is able to concentrate analytes and reactants, remove non-specifically bound molecules, provide stringency control for DNA hybridization reactions, and improve the detection of analytes. The device can be electronically replicated.

mode function, i.e., the underlying micro-electrode can still cause the rapid free field electrophoretic transport of other analyte molecules to or from the surface to which the oligonucleotide binding entities are attached. However, if large globular protein binding entities (e.g., antibodies) are attached to the same type of surface, they might insulate the surface and cause a decrease or a complete loss of the DC mode function. Appropriate modification of the attachment layer would have to be carried out so as to either reduce the number of large binding entities (e.g., large globular proteins) or provide spacing between the binding entities on the surface.

[0163] The spacing between microlocations is determined by the ease of fabrication, the requirement for detector resolution between microlocations, and the number of microlocations desired on a device. However, particular spacings between microlocations, or spacial arrangement or geometry of the microlocations is not necessary for device function, in that any combination of microlocations (i.e., underlying micro-electrodes) can operate over the complete device area. Nor is it actually necessary to enclose the device or completely confine the microlocations with dielectric or insulating barriers. This is because complex electronic field patterns or dielectric boundaries are not required to selectively move, separate, hold, or orient specific molecules in the space or medium between any of the electrodes. The device accomplishes this by attaching the specific binding molecules and subsequent analytes and reactants to the surface of an addressable microlocation. Free field electrophoretic propulsion provides for the rapid and direct transport of any charged molecule between any and all locations on the device; or from the bulk solution to microlocations. However, it should be pointed out that the devices might be enclosed for fluid containment and for bio-hazard purposes.

[0164] As the number of microlocations increases beyond several hundred, the complexity of the underlying circuitry of the microlocations increases. In this case the microlocation grouping patterns have to be changed and spacing distances increased proportionally, or multi-layer circuitry can be fabricated into the basic device, i.e., transistors and semiconductor control elements incorporated directly into the silicon.

[0165] In addition to microlocations which have been addressed with specific binding entities, a device will contain non-analytical microlocations and macrolocations which serve other functions. These microlocations or macrolocations can be used to store reagents, to temporarily hold reactants, analytes, or cells; and as disposal units for excess reactants, analytes, or other interfering components in samples (i.e., reagent dispensing and sample preparation systems). Other un-addressed microlocations can be used in combination with the addressed microlocations to affect or influence the reactions that are occurring at these specific microlocations. These microlocations add to both inter-device and intra-device activity and control. For example, a perimeter of microlocations (with underlying microelectrodes) surrounding an array of test site microlocations could be used as counter electrodes to encompass a large volume of test solution. Also, it is also possible for the microlocations to interact and transport molecules between two separate devices. This provides a mechanism for loading a working device with binding entities or reactants from a storage device, for sample preparations and for copying or replicating a device.

[0166] **FIG. 3** shows a matrix type device containing 64 addressable microlocations (30). A 64 microlocation device is a convenient design, which fits with standard microelectronic chip packaging components. Such a device is fabricated on a silicon chip substrate approximately 1.5 cm×1.5 cm, with a central area approximately 750 μm×750 μm containing the 64 microlocations. Each microlocation (**32**) is approximately 50 μm square with 50 μm spacing between neighboring microlocations. Connective circuitry for each individual underlying micro-electrode runs to an outside perimeter (10 mm×10 mm) of metal contact pads (300 μm square) (**34**). A raised inner perimeter can be formed between the area with the microlocations and the contact pads, producing a cavity which can hold approximately 2 to 10 microliters (μl) of a sample solution. The "chip" can be mounted in a standard quad package, and the chip contact pads (**34**) wired to the quad package pins. Systems containing more than one chip and additional packaging and peripheral components may be designed to address problems related to clinical diagnostics, i.e., addition of sample materials, fluid transfer, and containment of bio-hazardous materials. The packaged chip can then be plugged into a microprocessor controlled DC power supply and multimeter apparatus which can control and operate the device. It is contemplated by this invention that device manufacture (prior to addressing) will ultimately involve the incorporation of three basic components which would be essentially sandwiched together. The basic chip device to which the binding entities are attached, would be in the middle position; a sample or fluid containment component, would be annealed over the top and on board controller component would be annealed to the bottom of the basic chip device. This strategy solves a number of problems related to fabrication techniques and materials compatibilities.

[0167] I(b) Microlithography Fabrication Procedures

[0168] I(b)(1) Fabrication Steps General microlithographic or photolithographic techniques can be used for the fabrication of the complex "chip" type device which has a large number of small microlocations. While the fabrication of devices does not require complex photolithography, the selection of materials and the requirement that an electronic device function actively in aqueous solutions does require special considerations.

[0169] The 64 microlocation device (**30**) shown in **FIG. 3** can be fabricated using relatively simple mask design and standard microlithographic techniques. Generally, the base substrate material would be a 1 to 2 centimeter square silicon wafer or a chip approximately 0.5 millimeter in thickness. The silicon chip is first overcoated with a 1 to 2 μm thick silicon dioxide (SiO_2) insulation coat, which is applied by plasma enhanced chemical vapor deposition (PECVD).

[0170] In the next step, a 0.2 to 0.5 μm metal layer (e.g., aluminum) is deposited by vacuum evaporation. It is also possible to deposit metals by sputtering techniques. In addition to aluminum, suitable metals and materials for circuitry include gold, silver, tin, titanium, copper, platinum, palladium, polysilicon, carbon, and various metal combinations. Special techniques for ensuring proper adhesion to the

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